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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.
09/498,098	02/04/00	STACK J	AUR01330

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EXAMINER	
ANGELL, J	
ART UNIT	**PAPER NUMBER**
1633	9

DATE MAILED: 09/19/01

Please find below and/or attached an Office communication concerning this application or proceeding.

Commissioner of Patents and Trademarks

Office Action Summary	Application No.	Applicant(s)	
	09/498,098	STACK ET AL.	
	Examiner	Art Unit	
	Eirc Jon Angell	1633	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133).
- Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *04 February 2000* .

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-40,50,55 and 60* is/are pending in the application.

4a) Of the above claim(s) *55* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-40,50 and 60* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

11)☐ The proposed drawing correction filed on _____ is: a)☐ approved b)☐ disapproved by the Examiner.

If approved, corrected drawings are required in reply to this Office action.

12)☐ The oath or declaration is objected to by the Examiner.

Priority under 35 U.S.C. §§ 119 and 120

13)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____ .

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

14)☐ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. § 119(e) (to a provisional application).

a) ☐ The translation of the foreign language provisional application has been received.

15)☐ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. §§ 120 and/or 121.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO-1449) Paper No(s) _____ .
4) ☐ Interview Summary (PTO-413) Paper No(s). _____ .
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other:

DETAILED ACTION

Claims 1-40, 50, 55 and 60 are pending in the application.

Response to Amendment

The preliminary amendment filed on 2/4/2000 has been entered and claims 41-49, 51-54, 56-59 and 61-79 have been cancelled, as requested.

Election/Restrictions

1. Applicant's election without traverse of the claims of Group II (1-40, 50 and 60) drawn to methods of detecting an activity in a cell and recombinant nucleic acid molecules, limited to the species of mammalian cells, in Paper No. 8 filed 7/5/01 is acknowledged.

2. The claims of Group I (claims 1-40 and 55) drawn to methods of detecting an activity in a cell and recombinant proteins, limited to species of non-mammalian cells, are withdrawn from further consideration pursuant to 37 CFR 1.142(b) as being drawn to a nonelected invention. Claims 16-18, 29 and 30 are drawn to nonelected species and are therefore withdrawn from consideration. Election was made **without** traverse in Paper No. 8.

Claim Rejections - 35 USC § 102

3. The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless –
>
> (a) the invention was known or used by others in this country, or patented or described in a printed publication in this or a foreign country, before the invention thereof by the applicant for a patent.

4. Claim 38 is rejected under 35 U.S.C. 102(a) as being anticipated by Corish et al. (Protein Engineering 12 (12) 1035-1040. December, 1999).

Claim 38 is directed to a method of destabilizing a target protein in a cell comprising operatively coupling a target protein to a linear multimerized destabilization domain, wherein said destabilization domain is non cleavable by alpha-NH-ubiquitin protein endoproteases and comprises at least two copies of a destabilization domain.

Corish et al. disclose a method of destabilizing (i.e. decreasing the half-life) green fluorescent protein (GFP) in mammalian cells by operatively coupling a PEST domain to the C-terminus of GFP and a cyclin B1 destruction box to the N-terminus of said GFP (see abstract). The PEST domain and the cyclin B1 destruction box are capable of destabilizing GFP when individually coupled to GFP, but show an additive effect when both are operatively coupled to the target protein (see figure 2). The PEST domain and the destruction box act as destabilization domains and are not known to be cleavable by alpha-NH-ubiquitin protein endoproteases. The coupling of both the PEST domain and the destruction box to GFP is in essence coupling two copies of a destabilization domain (i.e. a linear multimerized destabilization domain) that is noncleavable by alpha-NH-ubiquitin protein endoproteases to a target protein. Therefore, Corish et al. clearly anticipate the method of claim 38.

Claim Rejections - 35 USC § 103

5. The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person

having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

6. Claims 38-40 are rejected under 35 U.S.C. 103(a) as being unpatentable over Johnson et al. (EMBO 11 (2) 497-505, 1992) in view of Hochstrasser (Ann. Rev. Genet. 30; 405-439, 1996).

Claims 38-40 are directed to a method of destabilizing a target protein in a cell comprising operatively coupling a target protein to a linear multimerized destabilization domain, wherein said destabilization domains are non cleavable by alpha-NH-ubiquitin protein endoproteases and comprises at least two copies of a destabilization domain, wherein the destabilization domain comprises a ubiquitin homolog and a mutation that prevents the cleavage by alpha-NH-ubiquitin protein endoproteases.

Johnson et al. (1992) teach a fusion protein comprising a linear ubiquitin domain containing an amino acid substitution at position 76 (i.e. Ub^{V76}-V-e^{K}-βgal; see Figures 1 VI and page 501, second paragraph) that prevents cleavage by alpha-NH-ubiquitin protein endoproteases. The mutant ubiquitin domain is fused in frame with a reporter moiety that results in a short *in vivo* half-life of the fusion protein (~5min.) (see page 497, second column; and page 501, second paragraph). Johnson et al. (1992) does not teach multimerization of the linear destabilization domain.

Hochstrasser teaches that addition of multiple ubiquitins to a proteolytic substrate, usually as a ubiquitin oligimer(s), appears to stimulate proteolysis, but monoubiquitination is clearly sufficient, at least *in vitro*, for appreciable rates of proteasomal degradation of some proteins, e.g. of alpha-globin in reticulocyte extracts. Hochstrasser also teaches degradation of some proteins is clearly accelerated by multiubiquitination *in vitro*, and this is likely to be true

for most of the substrates *in vivo* (see page 418, under Ubiquitin Chains). Therefore, it would have been obvious to an ordinary artisan at the time the claimed invention was made to combine the teachings of Johnson et al. and Hochstrasser and operatively couple a target protein to a linear multimerized and non-cleavable destabilization domain such as ubiquitin or a ubiquitin homolog, for the purpose of accelerating the degradation of a target protein such as a protein that is lethal at a high concentration, with a reasonable expectation of success.

7. Claim 50 is rejected under 35 U.S.C. 103(a) as being unpatentable over Hay et al. (WO 99/47640) in view of Johnson et al. (EMBO, 1992), further in view of Hochstrasser (Ann Rev Gen 1996).

Claim 50 is drawn to a recombinant DNA molecule, comprising a nucleic acid sequence encoding for a linear multimerized destabilization domain that is non-cleavable by alpha-NH-ubiquitin protein endoproteases, a target protein, and a linker moiety that operatively couples said multimerized destabilization domain to said target protein. The linker moiety is also non-cleavable by alpha-NH-ubiquitin protein endoproteases. The function of said DNA is to express the operatively coupled destabilization domain, linker and reporter moieties in a cell for purposes such as regulating the concentration of a target protein (i.e. a reporter), and for use in a method of detecting an activity, such a protease activity, in a cell.

Hay et al. teach a nucleic acid encoding a fusion protein compromising a reporter polypeptide linked to a linker polypeptide which is also linked to a repressor polypeptide. When the repressor polypeptide is operatively linked to the reporter polypeptide by the linker polypeptide, the result is repression of the activity of the reporter polypeptide (see claims 1 and

10). The linker polypeptide contains a protease cleavage site, such as a caspase cleavage site, and cleavage of the linker polypeptide results in an increase in the activity of the reporter polypeptide (see claims 1 and 2). The fusion protein is useful for purposes such as the detection of an activity, such as a protease activity, in a sample (see page 24 under "screening assays"). The fusion protein of Hay et al. in essence couples a reporter moiety to a repressor moiety using a linker moiety, resulting in repression of the activity of the reporter. The linker moiety regulates the coupling of the repressor moiety to the reporter moiety, and therefore can regulate the effect of the repressor moiety on the reporter moiety. Hay et al. also teaches that the linker contains a protease cleavage site, however, the protease cleavage site can be a caspase cleavage site. There is no evidence that caspase cleavage sites are cleavable by alpha-NH-ubiquitin protein endoproteases; therefore, a linker domain containing a caspase cleavage site would be noncleavable by alpha-NH-ubiquitin protein endoproteases. Hay et al. do not teach that the repressor polypeptide can be a multimerized destabilization domain.

Johnson et al. (1992) teach a fusion protein comprising a non-cleavable linear ubiquitin homolog (i.e. destabilization domain) fused in frame with a reporter moiety as mentioned in the 103(a) rejection above. Hochstrasser teaches degradation of some proteins is clearly accelerated by multiubiquitination, also as mentioned in the previous 103(a) rejection. Therefore, it would have been obvious to an ordinary artisan at the time the claimed invention was made to combine the teachings of Hay et al., Johnson et al., and Hochstrasser and construct a DNA molecule encoding a linear multimerized destabilization domain (e.g. ubiquitin or ubiquitin homologs) that is non-cleavable by alpha-NH-ubiquitin protein endoproteases, a target protein, and a linker moiety that is noncleavable by alpha-NH-ubiquitin protein endoproteases, wherein said linker

moiety operatively couples said destabilization domain and said reporter domain for the purpose of identifying molecules that modify the linker moiety with a reasonable expectation of success.

Claim Rejections - 35 USC § 112

8. The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

Claims 1-15, 19-28, 31-37 and 60 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for a method of detecting an activity in a cell, a method of regulating the concentration of a target protein in a cell, and a host cell comprising a nucleic acid sequence ***in vitro***, does not reasonably provide enablement for said methods and said cell ***in vivo***. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the invention commensurate in scope with these claims.

The claims as written read on both *in vitro* and *in vivo* embodiments. Although the specification discloses how the methods can be used *in vitro*, the state of the art of the production of transgenic animals at the time of filing was and continues to be unpredictable. For instance, it is well known in the art that the level and the specificity of expression of a transgene as well as the phenotype of a transgenic animal thus produced are greatly dependent on the specific transgene construct used. The individual gene of interest, promoter, enhancer, coding, or non-coding sequences present in the transgene construct, the site of integration, etc. are all important factors in controlling the expression of the transgene. It is well known that the transgenic art is

unpredictable in regard to producing animals of different species which exhibit identical phenotypes due to expression of a transgene. For example, Wall (Theriogenology, Vol. 45, pages 57-68, 1996) discloses the unpredictability of transgene behavior due to factors such as position effect and unidentified control elements resulting in a lack of transgene expression or variable expression (paragraph bridging pages 61-62). Overbeek ("Factors affecting transgenic animal production," Transgenic animal technology, pages 96-98, 1994) states that considerable variation in the level of transgene expression occurs between founder animals (page 96, last paragraph).

In addition, the species-specific requirements for transgene design are not clearly understood. Examples in the literature aptly demonstrate that even closely related species carrying the same transgene construct can exhibit widely varying phenotypes. For example, several animal models of human diseases have relied on transgenic rats when the development of mouse models was not feasible. Mullins et al. (1990) produced outbred Sprague-Dawley x WKY rats with hypertension caused by expression of a mouse *Ren-2* renin transgene. Hammer et al. (1990) describe spontaneous inflammatory disease in inbred Fischer and Lewis rats expressing human class I major histocompatibility allele HLA-B27 and human β_2-microglobulin transgenes. Both investigations were preceded by the failure to develop similar phenotypes in transgenic mice (Mullins et al., 1989; Taurog et al., 1988) expressing the same transgenes that successfully caused the desired symptoms in transgenic rats. Therefore, the phenotype of a theoretical transgenic animal was unpredictable at the time of filing and one of skill in the art could not readily predict that a transgenic animal would have the desired phenotype of interest.

The claims, as written, encompass transgenic non-human mammals to be used in a method of detecting an activity in a cell *in vivo*, a method of regulating the concentration of a target protein in a cell *in vivo*, and a host cell in the transgenic animal. The specification discloses possible methods of creating non-human transgenic. However, due to the examples stated above, the production of transgenic animals having the particular phenotype was unpredictable at the time of filing. One of skill in the art could not readily predict that any non-human transgenic animal would have the desired phenotype required to be useful for the methods claimed.

Therefore, in view of the quantity of experimentation necessary to determine the parameters listed above, the lack of direction and/or guidance in the specification, the absence of working examples for the methods claimed in all non-human organisms, the unpredictability of the art with respect to the expression of a transgene in all non-human mammals, and the breadth of the claims, it would have required undue experimentation for one skilled in the art to make and use the claimed invention with a reasonable expectation of success. The specification does discloses how an ordinary artisan could attempt to make a transgenic animal, however there could not be a reasonable expectation of success.

9. The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

10. Claims 22-28 and 31-37 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claim 22 recites the limitation "said test chemical". There is insufficient antecedent basis for this limitation in the claim because there is no recitation of "said test chemical" in the claim 20.

Claim 23 is unclear for the following reason. The preamble of the claim recites "a method of **regulating** the concentration of one or more target proteins in a cell..." (emphasis added), indicating that the method will allow for the control of both the increase and the decrease of the concentration of said protein. However, the method involves operatively coupling linear destabilization domain(s) to said target protein using a linker domain. Operatively coupling the destabilization domain(s) to the target protein results in the destabilization and degradation of said target protein. The linker domain contains a protease cleavage site such that when the linker domain is cleaved by said protease, the destabilization domain(s) is/are no longer operatively coupled to the target protein, resulting in an increase stability of the target protein and an increase in the concentration of the target protein. The claimed method, however, does not disclose how to decrease the concentration of said target protein. The claimed method can only result in the increase in concentration of the target protein; therefore, it is unclear and indefinite how the concentration of a target protein can be regulated (i.e. both increased and decreased) using the claimed method. Claims 23-28 and 31-37 depend on claim 22 and are therefore rejected for the same reason.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Jon Eric Angell whose telephone number is (703) 605-1165. The examiner can normally be reached on M-F (8:00-4:30).

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Deborah Clark can be reached on (703) 305-4051. The fax phone numbers for the organization where this application or proceeding is assigned are (703) 308-4242 for regular communications and (703) 308-4242 for After Final communications.

Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to the receptionist whose telephone number is (703) 308-0196.

J. E. Angell
September 10, 2001

REMY YUCEL, PH.D
PRIMARY EXAMINER